Influence of antimicrobials on starter cultures in milk – model trials

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Abstract

In addition to the toxicological significance of residues of antimicrobials in food from animals in the case of milk the technological aspect is of great economic importance. In model trials with yoghurt and mesophilic starter cultures for numerous antiinfectives those concentrations were derived from dose/response curves which caused defined inhibition effects with respect to the values of the corresponding negative controls (20% and 50% deviation in L(+)-, D(-)-lactate-, amino N-content, differences in pH value and bacterial counts). Most often, yoghurt cultures reacted more sensitive than the mesophilic starter culture. Significant differences to the corresponding negative control were stated with yoghurt culture for the following parameter at EU MRL concentrations: L(+)-lactate: penicillin, tylosin; D(-)-lactate: oxytetracycline, spiramycin, tylosin; amino N: penicillin, spiramycin; pH value: penicillin, cloxacillin, oxytetracyclin, spiramycin and tylosin.

From these experiments it can be concluded that starter cultures applied in dairy technology are influenced in the biochemical activity by residues of antibiotics on MRL level. Therefore, the testing of milk with methods capable of detecting residues of antimicrobials at least at or below the MRL level is necessary also from a technological aspect.

1 Introduction

The toxicological evaluation of residues of antimicrobials in food include pharmacological-toxicological, immunpathological and microbiological risks. The assessment of microbiological risks include mainly pathogenic microorganisms.

In case of the foodstuff milk besides the toxicological evaluation the aspect of technological safety is of great importance. The purpose of model trials with commercially available yoghurt culture was to evaluate whether the Maximum Residue Limits (MRLs) of antimicrobials which are fixed according to EU-Regulation 2377/90 ff ensure also technological safety.

Worldwide production of cheese and fermented milk products is at 14 mio metric tons and 100 mio metric tons per year, respectively. For this, approximately 2.5 mio tons of starters are needed (1). These figures indicate which large economic losses can occur if the activity of starter cultures is impaired by residues of anti-microbially active substances.

In this paper, model studies are described with two yoghurt cultures and one mesophilic starter culture with selected antimicrobially active substances in which, in a first trial, defined inhibitory activity is determined and in a second trial, the influences of residues at EU-MRL level are tested.

2 Experimental design

Antimicrobials: Penicillin G, ampicillin, cloxacillin, dicloxacillin, oxacillin, ceftiofur[1], dapson, sulfadimidine (= methazine), oxytetracycline , erythromycin, spiramycin, tylosin, DHstreptomycin, gentamicin, neomycin, enrofloxacin, trimethoprim.

Starter cultures: Yoghurt culture V 709 and V2 (Wiesby, Niebüll, Germany); mesophilic homofermentative culture, O-culture (R-607, Chr. Hansen's Laboratory, Lübeck, Germany).

Milk: Inhibitor free from the experimental herd of the Federal Dairy Research Centre; skimmed by centrifugation, heat-treated 15 min at 80°C, addition of 3 % mother culture, mixing and distribution into Erlenmeyer flasks, addition of antimicrobial/concentration desired, and bottling à 10 ml.

Incubation: In a waterbath at 42°C until the negative control reaches pH 4.5.

Sampling: Several times during incubation period, different for the measuring parameters

Parameter: pH value, L(+)-lactate (2), D(-)-lactate (2), amino N content (3), colony counts (M17, MRS) (4) and microscopic count (Breed) (5).

Experiment 1: Concentrations tested: 0, MRL, 10• MRL and 100• MRL; 1 trial.

Experiment 2: Concentrations tested: 0 and MRL; 3 independent trials.

3 Results

3.1 Experiment 1: Determination of defined inhibitory activity

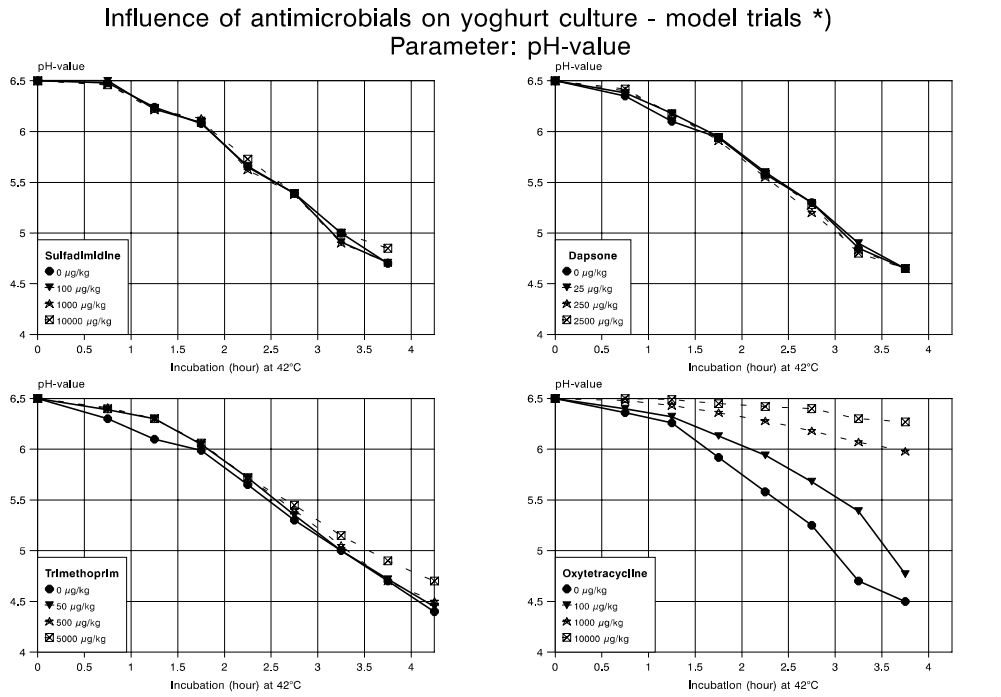
From **figure 1** it becomes evident that the chemotherapeutics sulfadimidine, dapsone and trimethoprim even in concentrations of 100• MRL do not have marked influence on the development of pH value. These substances do not seem to be of importance for technological reasons. In contrast to this oxytetracycline at MRL concentration is of pronounced influence on the development of the pH value; in milk with concentrations 10• - and 100• MRL the pH value just changed slightly during the incubation period.

Figure 2 demonstrates that as well β-lactam antibiotics (penicillin G, cloxacillin) as also the macrolides (spiramycin, tylosin) inhibited – under technological aspects – nearly totally the decrease of pH value at concentrations of 10• MRL. The influence of MRL levels differed for the tested substances.

Figure 3 shows the influence of penicillin G on bacterial counts. After an incubation period of 2.75 hours for all tested concentrations as well the number of streptococci as of lactobacilli was less than in the corresponding negative controls; at concentrations of 10• - and 100• MRL microscopic counts were much higher than colony counts.

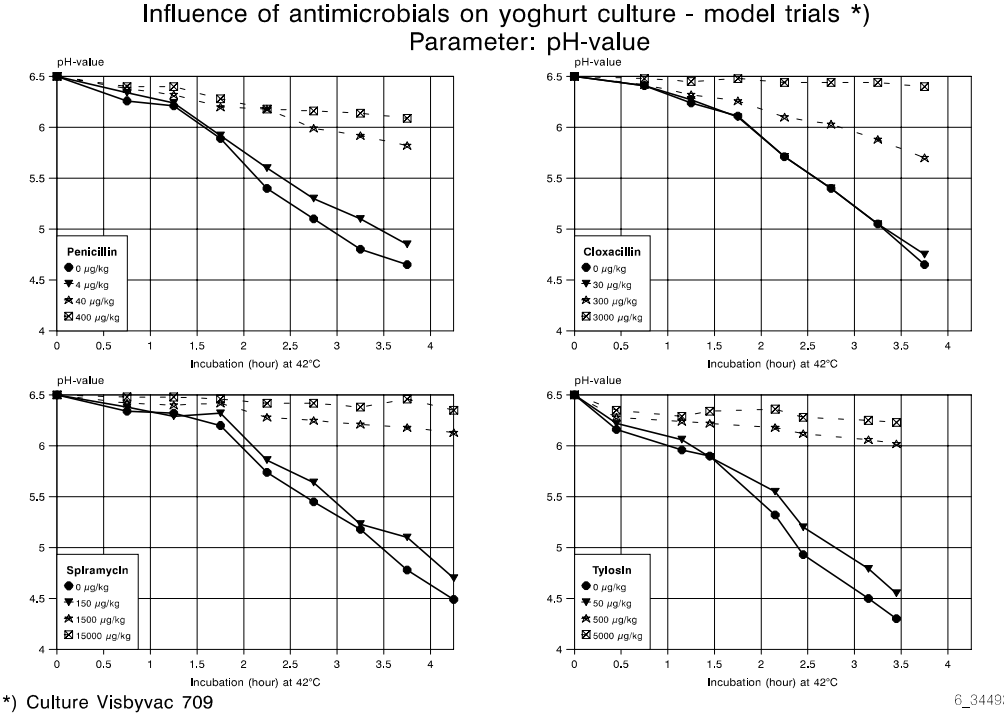
From graphs the lowest inhibitory concentrations – independent of the incubation period – are derived at which a marked inhibition (20 or 50 % respectively) compared to the negative control was observed. **Table 1** summarizes those antiinfectives and parameters at which inhibitory activities at < MRL level were observed. In particular with ceftiofur (only mother compound) and oxytetracycline, inhibition was observed at concentrations significantly below the MRLs.

[1] only mother compound



*) Culture Visbyvac 709

Figure 1



*) Culture Visbyvac 709

Figure 2

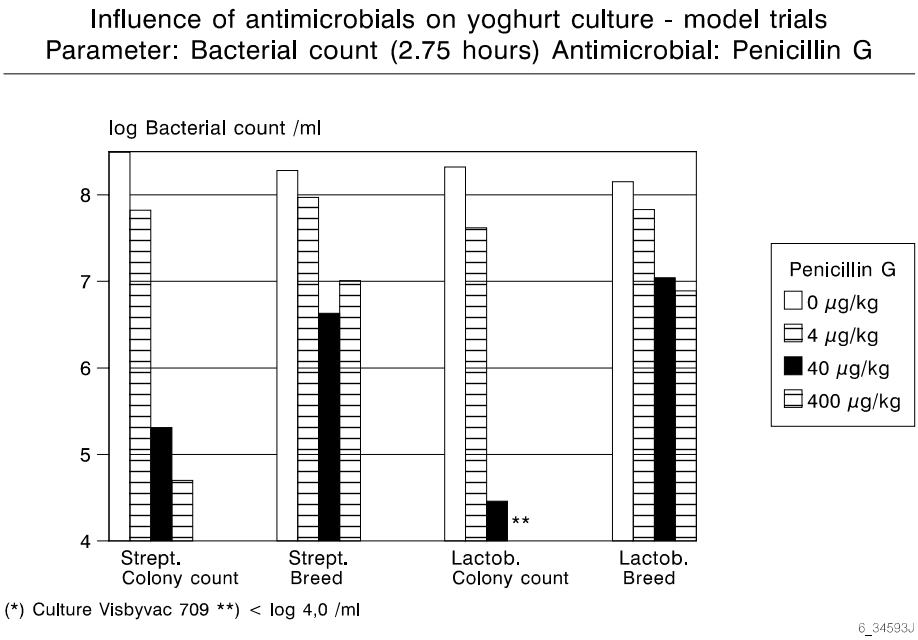


Figure 3

Table 1: Defined inhibition at ≤MRL level

Parameter	Yoghurt culture V2	Yoghurt culture V709	Mesophilic starter
L(+)-lactate	Ampicillin, oxacillin, ceftiofur[1)], oxytetracyline, erythromycin, spiramycin, tylosin	Penicillin, cloxacillin, oxytetracycline, spiramycin	Oxytetracycline
D(-)-lactate	Oxacillin, ceftiofur[1)], oxytetracyline, erythromycin, spiramycin, tylosin, gentamicin	Oxytetracyline, spiramycin, tylosin	
Amino N	Penicillin, ceftiofur[1)], oxytetracyline, erythromycin, spiramycin	penicillin, cloxacillin, oxytetracyline, spiramycin	Oxytetracyline, Tylosin
pH Value	Oxacillin, ceftiofur[1)], oxytetracyline, erythromycin, spiramycin	penicillin, cloxacillin, oxytetracyline, spiramycin, tylosin	Oxytetracyline
Streptococci	Ceftiofur[1)]		
Lactobacilli	Ceftiofur[1)]		

[1)] only mother compound

3.2 Experiment 2: Influence of antimicrobials at MRL level

At MRL concentration level, the tested chemotherapeutics – sulfadimidine, dapsone and trimethoprim – do not have a significant effect on starter cultures whereas for all tested antibiotics – penicillin, cloxacillin, oxytetracycline, spiramycin and tylosin – significant differences to the negative control were found depending on the antimicrobial, starter culture and measurement parameters. During the trials with yoghurt cultures, penicillin, for example, impaired especially L(+)-lactate formation (**fig. 4**) whereas spiramycin slowed down the D(-)-lactate formation (**fig. 5**). With the exception of oxytetracycline, yoghurt culture V709 proved to be more frequently significantly sensitive than yoghurt culture V2 and the mesophilic starter culture.

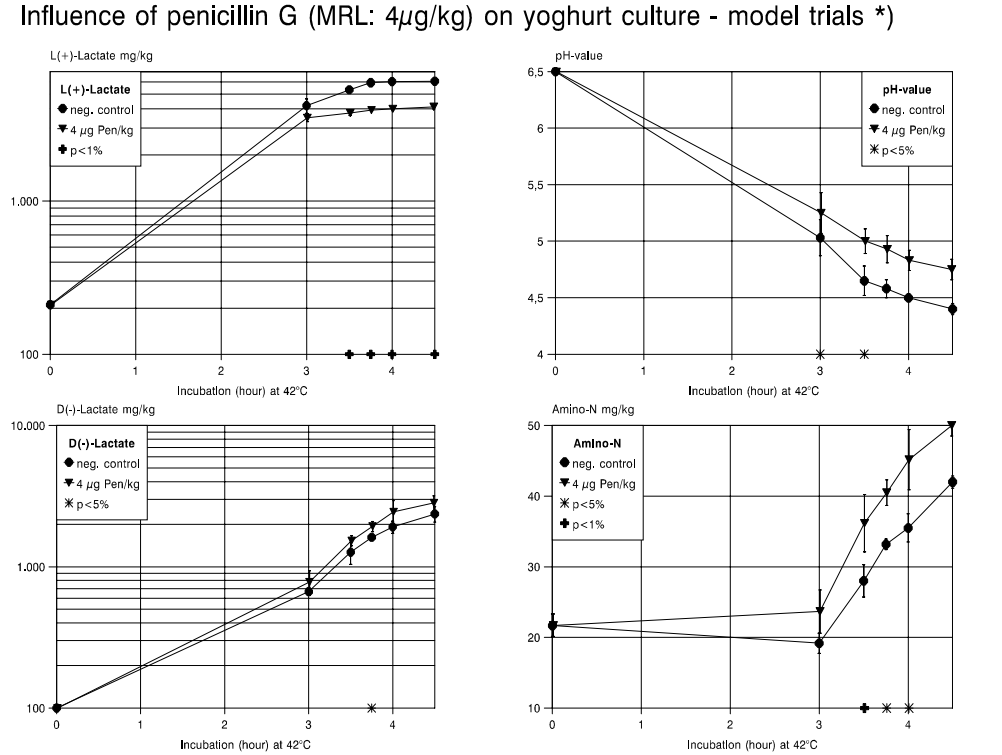


Figure 4

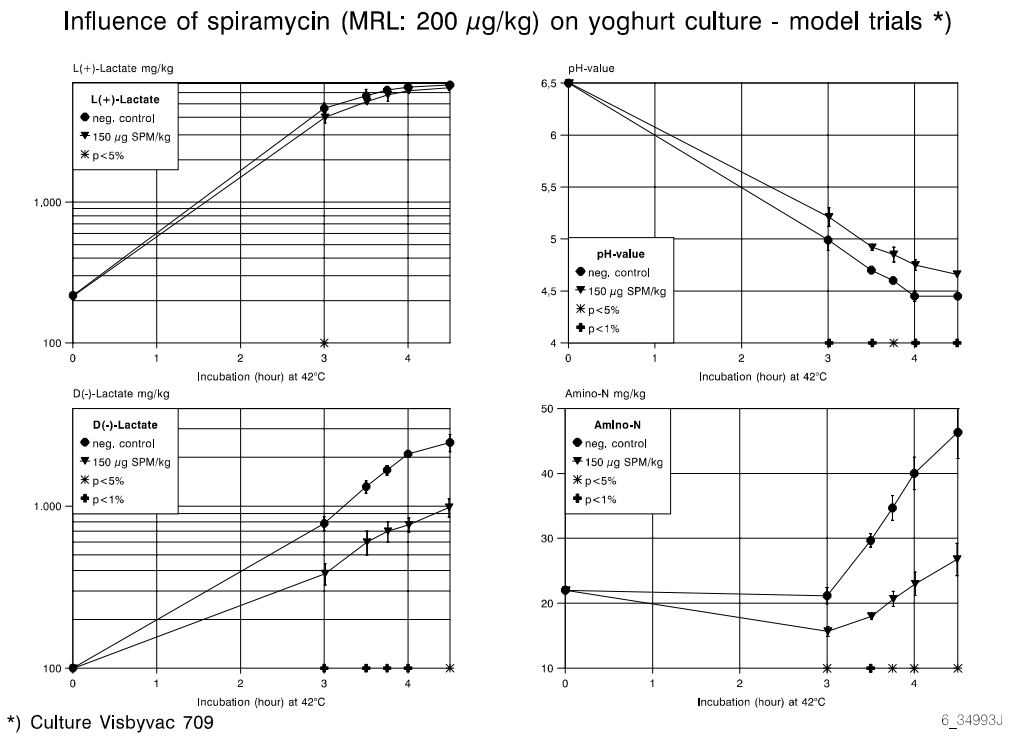


Figure 5

Conclusions

The model trials were carried out with two yoghurt and one mesophilic starter culture under standardized conditions. The inhibitory activities were defined, and therefore comparable, for these trials for different measurement parameters. They show highly specific results for the individually tested antiinfectives, and results that can be evaluated individually for all possible combinations.

In most cases, yoghurt cultures reacted more sensitive to residues of antiinfectives than the mesophilic starter cultures as also reported by Mäyrä-Mäkinen(1). An exception to this, for example, is the inhibition of the L(+)-lactate formation by residues of oxytetracycline: yoghurt culture V709 and the mesophilic starter show a comparable sensitivity regarding this parameter whereas the second tested yoghurt culture reacts significantly less sensitive to oxytetracycline.

The tested chemotherapeutics – sulfadimidine, trimethoprim, dapson and enrofloxacin – show an inhibition activity only at very high concentrations far above the MRL level. For mesophilic starter cultures, the L(+)-lactate and amino N formation as well as the reduction of the pH value are caused by a lower concentration of these chemotherapeutics than for yoghurt cultures.

Significant differences in comparison to the negative control were observed in milk containing antiinfectives at MRL concentrations for the parameter/antibiotic combinations listed in **Table 2**.

Table 2: Antiinfective/measurement parameters/culture combinations at which significant differences at MRL level were observed in reference to the negative control

	Yoghurt culture V709	Yoghurt culture V2	Mesophilic starters
L(+) lactate	Penicillin, tylosin	Penicillin, oxytetracyclin	Oxytetracyclin
D(-) lactate	Oxytetracyclin, spiramycin, tylosin	Oxytetracyclin, spiramycin, tylosin	not tested
Amino N	Penicillin, spiramycin	Spiramycin, tylosin	Oxytetracyclin
pH Value	Penicillin, cloxacillin, oxytetracyclin, spiramycin, tylosin	Oxytetracyclin, spiramycin	Cloxacillin, oxytetracyclin

Significant differences to antibiotic residues at MRL level were observed most often with yoghurt culture V709 whereas with the mesophilic starter culture, significant differences were observed almost only with oxytetracycline residues. Whereas cloxacillin at MRL level led to significant differences in only two cases (pH value with yoghurt culture V709 and mesophilic starter culture), penicillin, oxytetracycline, spiramycin and tylosin showed significant differences in comparison to the negative controls, which were tested in parallel, for numerous parameter/culture combinations.

From these experiments it can be concluded that starter cultures applied in dairy technology are influenced in their biochemical activity by residues of antibiotics at MRL level. Therefore, the testing of milk with methods capable of detecting residues of antimicrobials at least at or below the MRL level is necessary also from a technological aspect.

References

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